REMARKS

The drawings have been amended to add a figure 14A-B containing Table 1. The specification has been amended to provide a brief description of the new figure. The information in new Figure 14A-B and its accompanying description in the specification is exactly the same as the information in Table 1 of the application as filed. No new matter has been added.

Claims 64, 69, 73, 81, 85, 89, 97, 100, 103, 113, 114, 119, 120, 125, 130 and 135 have been amended to refer to Figure 14A-B, which contains Table 1. Claim 116 has been amended merely for clarity. No new matter has been added. Claims 64-79, 81-94, 96-108, 110-120, 122-125, 127-130 and 132-135 are pending.

Claim Objections

Claims 64-79, 81-94, 96-108, 110-115, 119, 12, 122-125, 127-130 and 12-135 are objected to because the base claims recite "Table 1." As suggested by the Examiner, the drawings have been amended to add Figure 14A-B containing Table 1 and the claims have been amended to recite Figure 14A-B, thereby obviating the objection.

Rejections Under 35 U.S.C. §112, First Paragraph

Claims 116-118 are rejected as "containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention." Claims 116-118 a recite a method for making a nucleic acid sequence that directs the synthesis of an optimized message. The method includes joining at least two synthesized, mRNA optimized fragments of a human protein of at least 90 amino acids. The Examiner asserts that:

> The specification does not specify which fragments of a nucleic acid sequence are joined together, and which adjoining portions of the protein are encoded by the fragments. There is no disclosure indicating the identities of the fragments of a

one skilled in the art would no know which portion of the protein is essential for function/activity and how to identify a functional protein.

In the interest of expediting prosecution, this rejection has been met by amending claim 116 to make clear that the recited optimized fragments encode contiguous portions of the human protein and that the optimized fragments are joined to create a nucleic acid encoding the human protein. It is clear that the claimed methods are directed to changing (optimizing) the nucleic acid encoding the protein, not the protein itself. Thus, the Examiner's comments about the identities of the portions of the protein and whether the encoded human protein is functional are not relevant to the present claims. The protein encoded by the resulting optimized message once the nucleic acid fragments are joined has the same structure and activity as the protein of interest to begin with. The protein of interest is not limited, and may be, e.g., a full length, fragment, or mutant human protein that is of interest to be expressed. Once the amino acid sequence of any human protein of interest is known, one of ordinary skill in the art could easily make optimized nucleic acids encoding the protein of interest according to the claimed methods. Moreover, at least one working example of the claimed methods is described in detail in the specification. See, e.g., page 45, line 1 to page 47, line 7, pages 48-55 and Figures 5 and 6, describing the optimization, according to the claimed methods, of a nucleic acid encoding a beta domain deleted Factor VIII. Therefore, the application as filed provides sufficient guidance for an ordinary artisan to practice the claimed methods.

Accordingly, Applicants respectfully request that the rejection be withdrawn.

Rejections under 35 U.S.C. §102

Claims 64-66, 68-71, 111 and 112 are rejected as anticipated by Kim et al. (Gene (1997) 199:293-301) (Kim). Claims 64-66, 68-71, 111 and 112 are directed to a synthetic nucleic acid sequence that encodes a protein where at least one non-common codon or less-common codon has been replaced by a common codon encoding the same amino acid residue as the non-common or less-common codon, and the synthetic nucleic acid sequence either: (a) includes a

the protein (claims 69-71). Claims 111-112 cover vectors and cells that include the aforementioned synthetic nucleic acids. The Examiner states that Kim et al. discloses the following:

> Two human erythropoietin (EPO) synthetic genes are generated, one gene (EPO^h) in which native codons were systematically substituted with codons frequently found in highly expressed human genes (Figs. 1 and 2; claims 64-66 and 68-71) and the other gene (EPO^y) with codons prevalent in yeast genes...

This rejection is respectfully traversed. Applicants have enclosed a marked-up copy of Fig. 2 from Kim in which non-common codons in the synthetic, mature EPO coding sequence of Kim (EPO^h) are circled. As can be seen from the marked-up figure, the EPO^h sequence does not include a contiguous stretch of at least 150 common codons or a continuous stretch of common codons which includes at least 60% or more of the total codons in the protein.

With regard to claims 64-66 and 68 (and related vector and cell claims 11 and 112), Kim et al. do not disclose or suggest an optimized nucleic acid (encoding EPO or any other protein) that includes a continuous stretch of at least 150 common human codons. The EPO^h coding sequence of Kim includes at least the following amino acids encoded by non-common human codons: P2, P3, R4, R14, C33, P87, P121, P122, and A124. For example, the most common codon encoding proline (P) in highly expressed human genes is CCC (see Fig. 1 of Kim and Table 1 of the present specification). However, the EPO coding sequence shown in Fig. 2 of Kim uses the codon CCA (P2, P3 and P87) and CCG (P121 and P122). Thus, Fig. 2 of Kim shows a synthetic EPO nucleic acid sequence where, at most, a continuous stretch of 53 codons is substituted with codons frequently found in highly expressed human genes (i.e., the continuous stretch between C33 and P87). Accordingly, Kim does not anticipate claims 64-66, 68 and the dependent vector and cell claims.

With respect to claims 69-71 (and related vector and cell claims 11 and 112), Kim et al. do not disclose or suggest an optimized nucleic acid (encoding EPO or any other protein) that includes a continuous stretch of common codons which includes at least 60% or more of the total codons in the protein. 60% of the mature EPO sequence (166 amino acids total) would be 100

frequently found in highly expressed human genes (i.e., the continuous stretch between C33 and P87). Accordingly, Kim does not anticipate claims 69-71 and the dependent vector and cell claims.

In light of the foregoing, Applicants respectfully request withdrawal of the rejection.

Additionally, Applicants reiterate the arguments made in the amendment and response filed on September 28, 2001, with respect to Kim. In sum, not only does Kim et al. not anticipate the claims, Kim in fact teaches away from the present invention by suggesting that a synthetic nucleic acid sequence should not have a high percentage of human common codons. Applicants direct the Examiner to Kim, page 299, last paragraph, where it is stated that:

> Re-engineered genes with human codon usage become high in their GC content. Although a low GC content of 5' UTR is ensured, optimizing the re-engineered gene further by decreasing the GC content of the limited region downstream of the initiator codon is advisable.

Far from decreasing the GC content of any region of the synthetic sequence, Applicants' presently claimed synthetic sequences have substantially increased GC content compared to the non-optimized sequence. Applicants found that construction of such synthetic sequences, contrary to the teachings of the art, provide a fruitful strategy for protein expression with disregard to CpG content. (See, e.g., Applicants' disclosure at page 43, lines 25-28). Applicants note that a rejection under 35 U.S.C. §103 over the Kim reference was overcome by the response filed on September 28, 2001.

Attached is a marked-up version of the changes being made by the current amendment.

Applicant asks the all claims be allowed. The Examiner is respectfully requested to telephone the undersigned Applicants' representative if any issues remain.

Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 12 Dec. 00

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Version with markings to show changes made

In the claims:

Claims 64, 69, 73, 81, 85, 89, 97, 100, 103, 113, 114, 116, 119, 120, 125, 130 and 135 have been amended as follows:

64. (Amended) A synthetic nucleic acid sequence which encodes a human protein wherein at least one non-common codon or less-common codon has been replaced by a common codon encoding the same amino acid residue as the non-common or less-common codon, and the synthetic nucleic acid sequence comprises a continuous stretch of at least 150 codons all of which are common codons, wherein by a common codon is meant the most common codon encoding each particular amino acid residue in highly expressed human genes as shown in [Table 1] Figure 14A-B.

69. (Amended) A synthetic nucleic acid sequence which encodes a human protein wherein at least one non-common codon or less-common codon has been replaced by a common codon encoding the same amino acid residue as the non-common or less-common codon, and the synthetic nucleic acid sequence comprises a continuous stretch of common codons, which continuous stretch includes at least 60% or more of the codons in the synthetic nucleic acid sequence, wherein by a common codon is meant the most common codon encoding each particular amino acid residue in highly expressed human genes as shown in [Table 1] Figure 14A-B.

73. (Amended) A synthetic nucleic acid sequence which encodes a human protein wherein at least one non-common codon or less-common codon has been replaced by a common codon encoding the same amino acid residue as the non-common or less-common codon, and wherein at least 98% or more of the codons in the sequence encoding the protein are common codons and wherein the protein is at least 90 amino acid residues in length, wherein by a common codon is meant the

81. (Amended) A synthetic nucleic acid sequence which encodes human Factor VIII or a functional portion thereof, wherein at least one non-common codon or less-common codon has been replaced by a common codon encoding the same amino acid residue as the non-common or less-common codon and wherein the synthetic nucleic acid has a continuous stretch of at least 150 codons all of which are common codons, wherein by a common codon is meant the most common codon encoding each particular amino acid residue in highly expressed human genes as shown in [Table 1] Figure 14A-B.

85. (Amended) A synthetic nucleic acid sequence which encodes human Factor VIII or a functional portion thereof, wherein at least one non-common codon or less-common codon has been replaced by a common codon encoding the same amino acid residue as the non-common or less-common codon and wherein the synthetic nucleic acid has a continuous stretch of common codons which comprise at least 60% of the codons of the synthetic nucleic acid sequence, wherein by a common codon is meant the most common codon encoding each particular amino acid residue in highly expressed human genes as shown in [Table 1] Figure 14A-B.

89. (Amended) A synthetic nucleic acid sequence which encodes human Factor VIII or a functional portion thereof, wherein at least one non-common codon or less-common codon has been replaced by a common codon encoding the same amino acid residue as the non-common or less-common codon and wherein at least 98% or more of the codons in the sequence encoding the Factor VIII are common codons and the Factor VIII is at least 90 amino acid residues in length, and wherein by a common codon is meant the most common codon encoding each particular amino acid residue in highly expressed human genes as shown in [Table 1] Figure 14A-B.

97. (Amended) A synthetic nucleic acid sequence which encodes human Factor IX, wherein at least one non-common codon or less-common codon has been replaced by a common

[illegible] wherein by a common codon is meant the most common codon encoding

each particular amino acid residue in highly expressed human genes as shown in [Table 1] Figure 14A-B.

100. (Amended) A synthetic nucleic acid sequence which encodes human Factor IX, wherein at least one non-common codon or less-common codon has been replaced by a common codon encoding the same amino acid residue as the non-common or less-common codon and wherein the synthetic nucleic acid has a continuous stretch of common codons which comprise at least 60% of the codons of the synthetic nucleic acid sequence, and wherein by a common codon is meant the most common codon encoding each particular amino acid residue in highly expressed human genes as shown in [Table 1] Figure 14A-B.

103. (Amended) A synthetic nucleic acid sequence which encodes human Factor IX, wherein at least one non-common codon or less-common codon has been replaced by a common codon encoding the same amino acid residue as the non-common or less-common codon and wherein at least 98% or more of the codons in the sequence encoding the Factor IX are common codons and the Factor IX is at least 90 amino acid residues in length, and wherein by a common codon is meant the most common codon encoding each particular amino acid residue in highly expressed human genes as shown in [Table 1] Figure 14A-B.

113. (Amended) A synthetic nucleic acid sequence which encodes a human protein wherein at least one non-common codon or less-common codon has been replaced by a common codon encoding the same amino acid residue as the non-common or less-common codon, wherein by a common codon is meant the most common codon encoding each particular amino acid residue in highly expressed human genes as shown in [Table 1] Figure 14A-B and having the following properties:

(i) the synthetic nucleic acid sequence comprises a continuous stretch of at least 150 codons all of which are common codons;

(ii) the synthetic nucleic acid

(iii) wherein at least 98% or more of the codons in the sequence encoding the protein are common codons and wherein the protein is at least 90 amino acid residues in length.

114. (Amended) A method for preparing a synthetic nucleic acid sequence which is at least 90 codons in length, comprising:

identifying a non-common codon and a less-common codon in a non-optimized gene sequence which encodes a human protein and is at least 90 codons in length; and

replacing at least 98% of the non-common and less-common codons with a common codon encoding the same amino acid residue as the replaced codon, wherein by a common codon is meant the most common codon encoding each particular amino acid residue in highly expressed human genes as shown in [Table 1] Figure 14A-B.

116. (Amended) A method for making a nucleic acid sequence which directs the synthesis of an optimized message of a human protein of at least 90 amino acids comprising:

synthesizing at least two fragments of a nucleic acid sequence, wherein the two fragments encode [adjoining] contiguous portions of a human protein of at least 90 amino acids and wherein both fragments are mRNA optimized; and

joining the two fragments to create a nucleic acid encoding the human protein, such that a non-common codon is not created at a junction point, thereby making the mRNA optimized nucleic acid sequence.

119. (Amended) A method for preparing a synthetic nucleic acid sequence encoding a human protein which is at least 90 amino acid residues in length, comprising identifying non-common codon and less-common codons in the non-optimized nucleic acid sequence encoding a protein of at least 90 amino acid residues in length and replacing at least 98% or more of the non-common and less-common codons of the nucleic acid sequence encoding the protein with a common codon encoding the same amino acid residue as the replaced codon, wherein by a

synthetic nucleic acid sequence encoding a human protein which is at least 90 amino acid residues in length.

120. (Amended) A primary or secondary mammalian cell having an exogenous synthetic nucleic acid sequence which encodes a human protein or a polypeptide wherein at least one non-common codon or less-common codon has been replaced by a common codon encoding the same amino acid residue as the non-common or less-common codon, wherein by a common codon is meant the most common codon encoding each particular amino acid residue in highly expressed human genes as shown in [Table 1] Figure 14A-B, and wherein the synthetic nucleic acid has a continuous stretch of at least 150 codons all of which are common codons; is at least 80 base pairs in length; and is free of unique restriction endonuclease sites in the message optimized sequence; and has

DNA sequences, sufficient for expression of the exogenous synthetic DNA in the transfected primary or secondary cell;

the primary or secondary cell capable of expressing the human protein or polypeptide product.

125. (Amended) A primary or secondary mammalian cell having an exogenous synthetic nucleic acid sequence which encodes a human protein or a polypeptide wherein at least one non-common codon or less-common codon has been replaced by a common codon encoding the same amino acid residue as the non-common or less-common codon, wherein by a common codon is meant the most common codon encoding each particular amino acid residue in highly expressed human genes as shown in [Table 1] Figure 14A-B, and wherein the synthetic nucleic acid has a continuous stretch of common codons which comprise at least 60% of the codons of the synthetic nucleic acid sequence, is at least 80 base pairs in length and is free of unique restriction endonuclease sites in the message optimized sequence; and has

DNA sequences, sufficient for expression of the exogenous synthetic DNA in the

130. (Amended) A primary or secondary mammalian cell having an exogenous synthetic nucleic acid sequence which encodes a human protein or a polypeptide wherein at least one non-common codon or less-common codon has been replaced by a common codon encoding the same amino acid residue as the non-common or less-common codon, wherein by a common codon is meant the most common codon encoding each particular amino acid residue in highly expressed human genes as shown in [Table 1] Figure 14A-B, and wherein at least 98% or more of the codons in the sequence encoding the protein are common codons and the protein is at least 90 amino acids in length; the nucleic acid sequence is at least 80 base pairs in length and is free of unique restriction endonuclease sites in the message optimized sequence; and has

DNA sequences, sufficient for expression of the exogenous synthetic DNA in the transfected primary or secondary cell;

the primary or secondary cell capable of expressing the human protein or polypeptide product.

135. (Amended) A primary or secondary mammalian cell having an exogenous synthetic nucleic acid sequence which encodes a human protein or a polypeptide wherein at least one non-common codon or less-common codon has been replaced by a common codon encoding the same amino acid residue as the non-common or less-common codon, wherein by a common codon is meant the most common codon encoding each particular amino acid residue in highly expressed human genes as shown in [Table 1] Figure 14A-B, and wherein the synthetic nucleic acid has the following properties: it has a continuous stretch of at least 150 codons all of which are common codons; it has a continuous stretch of common codons which comprise at least 60% of the codons of the synthetic nucleic acid sequence; at least 98% or more of the codons in the sequence encoding the protein are common codons and the protein is at least 90 amino acids in length; it is at least 80 base pairs in length and which is free of unique restriction endonuclease sites in the message optimized sequence; and

DNA

the primary or secondary cell capable of expressing the human protein or polypeptide product.

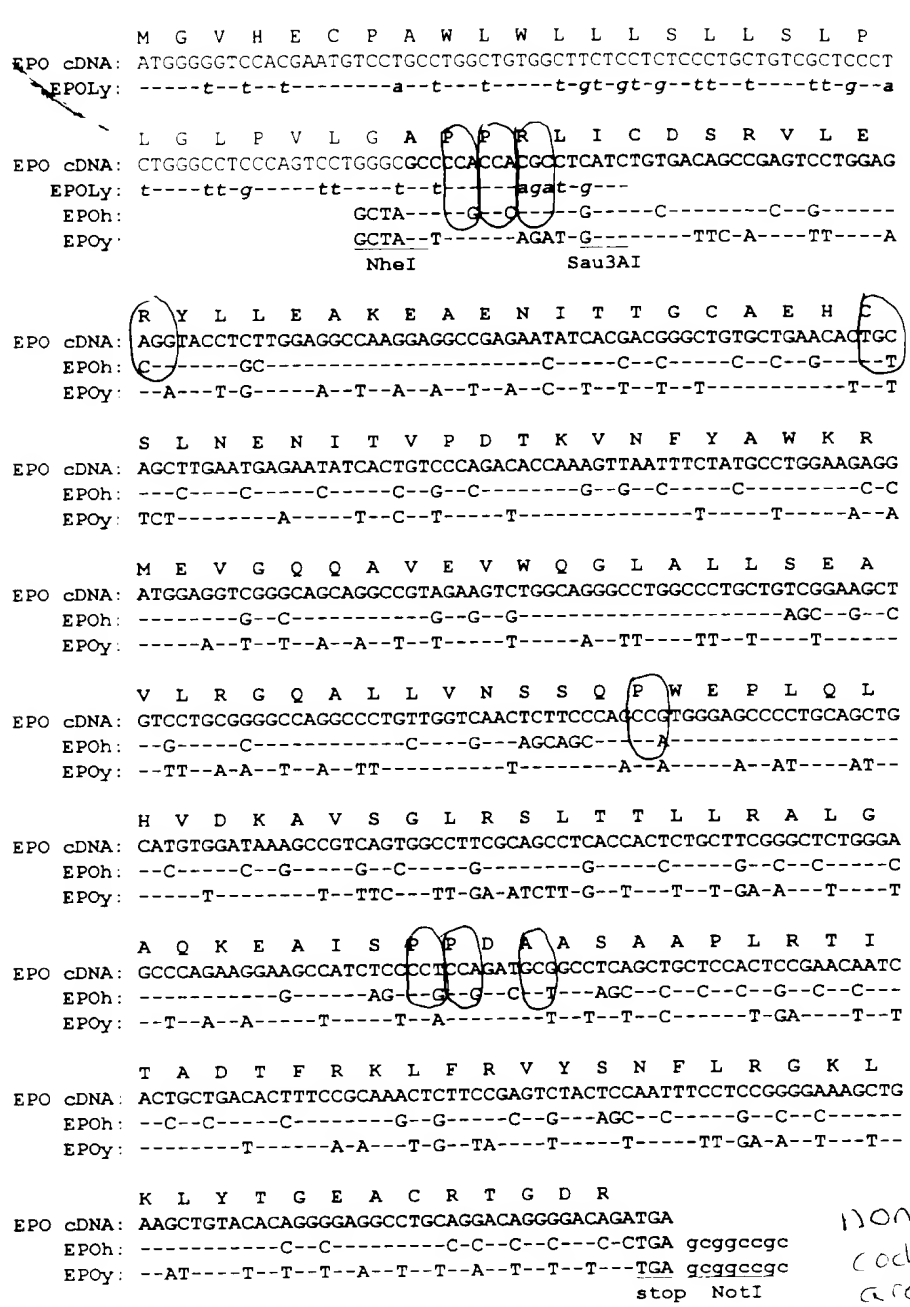

Fig. 2. Nucleotide sequence of the EPO cDNA and the mature EPO genes with human and yeast codon usage (*EPO*^{h} and *EPO*^{y}). The deduced amino acid sequence shown above each codon is designated by the single letter code. Nucleotide and amino acid sequences of mature EPO are shown in bold. The substituted nucleotides of the synthetic mature EPO genes (*EPO*^{h} and *EPO*^{y}) are shown below the EPO cDNA sequence in two lines. The italicized nucleotides indicate the yeast codon-based synthetic sequence encoding the EPO leader peptide and consecutive six amino acids (*EPOL*^{y}). The sites of the restriction enzymes used for cloning are also indicated above.

EPO gene were separately generated by the first PCR. Typically, PCR was conducted using 30 cycles with an annealing temperature of 50°C and 30-s extension time, which contained a sequence for a restriction site (*Nhe*I or *Not*I) and an adjacent sequence complementary to the 5′ or 3′ end of the mature EPO gene, were subse-

TABLE 1

Codon Frequency in Highly Expressed Human Genes

		% occurrence			% occurrence			% occurrence
Glu			Cys			Gln		
GA	A	25	TG	C	68	CA	A	12
	G	75		T	32		G	88
Arg			Ala			Gly		
CG	C	37	GC	C	53	GG	C	50
	T	7		T	17		T	12
	A	6		A	13		A	14
	G	21		G	17		G	24
AG	A	10						
	G	18						
Leu			Ser			Pro		
CT	C	26	TC	C	28	CC	C	48
	T	5		T	13		T	19
	A	3		A	5		A	16
	G	58		G	9		G	17
TT	A	2	AG	C	34			
	G	6		T	10			

TABLE 1 (cont.)

Codon Frequency in Highly Expressed Human Genes

	% occurrence			% occurrence			% occurrence		
Ilc			Thr			Val		
AT	C	77	AC	C	57	GT	C	25
	T	18		T	14		T	7
	A	5		A	14		A	5
				G	15		G	64
Tyr			Phe			Lys		
TA	C	74	TT	C	80	AA	A	18
	T	26		T	20		G	82
Asn			His					
AA	C	78	CA	C	79			
	T	25		T	21			

FIGURE 14B